

Environmental Effects on the Growth, Maturation and Physiology in Antarctic Krill (*Euphausia superba*) Over an Annual Cycle: An Experimental Approach



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-- Declaration --

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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-- Abstract --

Antarctic krill, *Euphausia superba*, is a keystone species in the Antarctic ecosystem, being a major food source for most predators and the target of a substantial fishery. Despite being a critical component in the Southern Ocean, limited information exists on krill growth, maturation and physiology under various light, diet and temperature regimes throughout a full year. Without a comprehensive understanding of these factors, forecasting adaptations in a changing environment is hampered. This study examines the effects of the key environmental parameters (light, food availability and temperature) on growth, maturation and physiology in krill.

Krill were incubated for an annual cycle under natural light (emulating the field environment), and constant food supply and temperature. Krill showed a clear seasonal cycle of growth and maturity in all three temperature treatments (-1°C , 1°C , 3°C). Sex significantly affected the relationship with growth over a year. Overall, females showed higher growth rates than males, and growth rapidly decreased after the peak growth period towards the end of spring. Males peaked in growth and matured earlier than females and decreased growth at a considerably slower rate. Negative growth occurred towards the end of January for both sexes, coinciding with the regression of external sexual characteristics. There was a significant decline in intermoult period (IMP) with increasing temperature and some evidence to suggest that 1°C was optimum for krill growth. The IMP was significantly lower at 1°C than at -1°C , but the difference in growth increment (GI) between the two temperatures was not significantly different, with all growth variables significantly lower at 3°C . For the first time, this study has confirmed that compensation mechanisms do exist between IMP and instantaneous growth rate (IGR) for krill, resulting in short IMP/small IGR to long IMP/large IGR.

Based on external sexual characteristics (female – thelycum; male – petasma), krill exposed to a natural Antarctic light cycle or a fixed light/dark regime, progress under a natural maturation cycle of regression and re-maturation. However, when krill were maintained in complete darkness during sexual regression, the rate of regression accelerated and re-maturation occurred three months earlier in the following season. This flexible maturation cycle in response to conditions of total darkness at the time of regression means that krill can flexibly adjust their seasonal physiological cycle. Overall, light (in this case darkness) appears to be one of the most important factors influencing the krill maturation cycle.

There was a strong significant increasing trend of respiration rates in krill with month in all experimental conditions; natural light cycle versus complete darkness, fed versus starved and different temperature regimes (-1°C , 1°C and 3°C). The interaction of treatment with month, as well as generally the main effect of each treatment, was non-significant. Overall, from this study, it appears that light, food availability and temperature may not be the dominant environmental variables influencing the observed seasonal changes in metabolic rates.

There was no significant difference throughout the year (except February) in total lipid and fatty acid content and composition of immature krill, and also between mature males and females in summer. The lipid and fatty acid concentrations were near depletion in February for all krill, indicating these reserves were possibly used for reproductive purposes rather than as an overwintering source. Mated females were only observed at -1°C in November. Lipid and fatty acid levels were lower in mated compared to un-mated females, indicating utilisation of lipids during the mating process. There was no clear temperature effect on lipid and fatty acid content and composition at the various time points sampled; however, krill at the lower temperature, -1°C , generally contained higher lipid and fatty acid content.

This study has provided a solid basis for understanding the life history of krill over an annual cycle, which will enable more robust modelling for accurate assessments and management for the krill fishery. This research has further helped elucidate the effects of key environmental parameters on the growth, maturation and physiology in krill. It is crucial to expand on this knowledge so as to comprehend seasonal adaptation and survival of krill in a changing habitat, in light of predicted climatic change.

-- Table of Contents --

Declaration.....	ii
Abstract.....	iii
Table of Contents.....	v
Acknowledgments	viii
Co-Authorship	x
Acronyms and Abbreviations	xii

Chapter one: General Introduction 1

1.1. Overview of Antarctic krill.....	1
1.1.1. Food chain.....	2
1.1.2. Krill fishery and management.....	4
1.2. Methodology of past research.....	5
1.2.1. Field studies	5
1.2.2. Laboratory studies	6
1.3. Krill growth.....	7
1.4. Maturation and reproduction on krill.....	10
1.4.1. Environmental effects of reproductive processes	12
1.5. Overwintering processes.....	14
1.6. Climatic change	17
1.7. Aims and objectives.....	19
1.8. Research approach	19

Chapter two: Temperature effects on the growth and maturation of Antarctic krill (*Euphausia superba*) 21

2.1. Abstract	21
2.2. Introduction.....	22
2.3. Materials and methods	24
2.3.1. Sampling and experimental setup.....	24
2.3.2. Experimental procedure	25
2.3.3. Statistical analysis	26
2.4. Results	27
2.4.1. Seasonal growth cycle.....	28
2.4.2. Seasonal maturity cycle.....	28
2.4.3. Linear mixed model predictions.....	33
2.4.3.1. Effect of temperature and total length	33
2.4.3.2. Effect of month	36
2.4.3.3. Between- and within-animal variance	40
2.4.3.4. Assessment of tank effect	41
2.4.3.5. Normality	41
2.5. Discussion	42
2.5.1. Intermoult period.....	42
2.5.2. Daily growth rates.....	44
2.5.2.1. Temperature effect.....	44
2.5.2.2. Effect of season and sex.....	46
2.5.3. Differential growth rates between sexes	46
2.5.4. Consideration of experimental effect.....	47
2.5.5. Variance and compensation of growth.....	48
2.6. Conclusion.....	48

Chapter three: Flexible adaptation of the seasonal krill maturation cycle in the laboratory	50
<u>3.1. Abstract</u>	50
<u>3.2. Introduction</u>	51
<u>3.3. Materials and methods</u>	53
3.3.1. Collection of krill	53
3.3.2. Aquaria conditions	53
3.3.3. Experimental procedure	54
3.3.3.1. Rationale of light regimes	55
3.3.4. Determination of sexual maturity stages	56
<u>3.4. Results</u>	57
<u>3.5. Discussion</u>	58
<u>3.6. Conclusion</u>	62
 Chapter four: Long-term effect of photoperiod, temperature and feeding regimes on the respiration rates of Antarctic krill (<i>Euphausia superba</i>) in the laboratory	63
<u>4.1. Abstract</u>	63
<u>4.2. Introduction</u>	64
<u>4.3. Materials and methods</u>	66
4.3.1. Collection of krill	66
4.3.2. Aquaria conditions	66
4.3.3. Measurement of respiration rates	68
4.3.4. Statistical analysis	68
<u>4.4. Results</u>	68
<u>4.5. Discussion</u>	76
4.5.1. Food availability	76
4.5.2. Temperature	77
4.5.3. Photoperiod	78
4.5.4. Endogenous annual rhythm	79
<u>4.6. Conclusion</u>	80
 Chapter five: Effects of temperature and constant food supply on immature Antarctic krill (<i>Euphausia superba</i>) over a full year: Lipid and fatty acid content and composition	81
<u>5.1. Abstract</u>	81
<u>5.2. Introduction</u>	82
<u>5.3. Materials and methods</u>	84
5.3.1. Sampling and experimental setup	84
5.3.2. Lipid extraction	86
5.3.3. Lipid class analysis	86
5.3.4. Fatty acid analysis	86
5.3.5. Statistical analysis	87
<u>5.4. Results</u>	87
5.4.1. Total lipid content and class composition	87
5.4.2. Fatty acid content composition	90
<u>5.5. Discussion</u>	95
5.5.1. Seasonal variation in total lipid content and composition	95
5.5.2. Seasonal variation in fatty acid content and composition	98
5.5.3. Temperature effects	99
<u>5.6. Conclusion</u>	100

Chapter six: Effects of temperature and constant food supply on lipid and fatty acid content and composition with respect to sex and body tissue of Antarctic krill (*Euphausia superba*) in summer 102

6.1. Abstract	102
6.2. Introduction	103
6.3. Materials and methods	105
6.3.1. Sampling and experimental setup	105
6.3.2. Lipid extraction	106
6.3.3. Lipid class analysis	106
6.3.4. Fatty acid analysis	107
6.3.5. Statistical analysis	107
6.4. Results	107
6.4.1. Total lipid content and class composition	107
6.4.1.1. November	108
6.4.1.1.1. Lipid content in thoracic components and digestive gland	108
6.4.1.1.2. Relative levels of lipid classes	110
6.4.1.2. February	110
6.4.1.2.1. Lipid content in thoracic component	110
6.4.1.2.2. Lipid content in digestive gland	113
6.4.1.2.3. Relative levels of lipid classes	113
6.4.2. Fatty acid content and composition	113
6.4.2.1. November	114
6.4.2.1.1. Sex related differences in fatty acid content	114
6.4.2.1.2. Temperature related differences in fatty acid content	114
6.4.2.2. February	114
6.4.2.2.1. Sex related differences in fatty acid content	114
6.4.2.2.2. Temperature related differences in fatty acid content	117
6.4.2.3. Relative levels of fatty acids for November and February	117
6.4.2.3.1. Sex related differences	117
6.4.2.3.2. Temperature related differences	118
6.5. Discussion	122
6.5.1. Sex related differences	122
6.5.1.1. Effect of experimental condition	124
6.5.2. Distribution of lipids	125
6.5.3. Temperature related differences	126
6.6. Conclusion	128

Chapter seven: General Discussion..... 129

7.1. Temperature effects on growth and maturity	129
7.2. Effect of various light regimes on the maturation cycle	130
7.3. Seasonal variation in respiration rates	131
7.4. Trends in lipid content and composition	132
7.5. Consideration of the effects of experimental conditions	132
7.6. Endogenous annual rhythm	134
7.7. Evidence of climate change	136
7.8. Optimal temperature for krill growth, maturation and condition	136
7.9. Other parameters influenced by global warming	137
7.10. Comparison studies to krill from the warmer waters at South Georgia	138
7.11. Physiological and behavioural plasticity of other euphausiids	140
7.12. Conclusion	142

Literature cited **143**

Appendices **160**

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- ii. **Brown, M.**, Kawaguchi, S., King, R., Virtue, P. and Nicol, S. Flexible adaptation of the seasonal krill maturation cycle in the laboratory. *Submitted to Journal of Plankton Research*. —————→ **Chapter 3**

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- iv. **Brown, M.**, Virtue, P., Nichols, P., Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on immature Antarctic krill (*Euphausia superba*) over a full year: Lipid and fatty acid content and composition. *Submitted to Comparative Biochemistry and Physiology, Part B*. —————→ **Chapter 5**

- v. **Brown, M.**, Virtue, P., Nichols, P. and Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on lipid and fatty acid content and composition with respect to sex and body tissue of Antarctic krill (*Euphausia superba*) in summer. *Submitted to Comparative Biochemistry and Physiology, Part B*. —————→ **Chapter 6**

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- The concept and design of this thesis was developed by M. Brown and S. Kawaguchi.
- S. Kawaguchi, P. Virtue, P. Nichols and S. Nicol assisted with the general supervision of this thesis. This included experimental design, general advice, interpretation of data, and proof reading and contributing to the above listed chapters/manuscripts.
- All laboratory experiments and measurements in this thesis were conducted by M. Brown. In Chapter 4, some of the laboratory measurements were performed by T. Yoshida. Laboratory assistance was provided by P. Virtue and P. Nichols with lipid and fatty acid analyses in Chapters 5 and 6.
- Statistical analysis in Chapter 2 and 4 was conducted by S. Candy. Statistics in the remainder of the thesis was performed by M. Brown, with advice from S. Candy.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above submitted peer-reviewed chapters/manuscripts contributing to this thesis.



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-- Acronyms and Abbreviations --

Definitions of the main acronyms used throughout the thesis:

Acronym	Definition
°C	Degrees Celcius
AA	Arachidonic acid (20:4 ω 6)
AAD	Australian Antarctic Division
ANOVA	Analysis of variance
BAV	Between-animal variance
CCAMLR	Commission for (and Convention on) the Conservation of Antarctic Marine Living Resources
Chl a	Chlorophyll a
DAG	Diacylglycerol
DHA	Docosahexaenoic acid (22:6 ω 3)
DGR	Daily growth rate (mm day ⁻¹)
DW	Dry weight (mg)
EPA	Eicosapentaenoic acid (20:5 ω 3)
F	Female
FA	Fatty acid
FAME	Fatty acid methyl esters
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometer
GI	Growth increment in total length (mm)
H1	Holding tank 1
H2	Holding tank 2
HC	Hydrocarbon
hr	Hour
IGR	Instantaneous growth rate (%)
ind	Individual
IMP	Intermoult period (days)
L	Litre
LA	Linoleic acid (18:2 ω 6)
LMM	Linear mixed model
M	Male
m	Metre
mL	Milli-Litre
month.f	Month as a factor, with a set of integer values ranging between 4-17 (Apr 06 – May 07)
MUFA	Monounsaturated fatty acid
MS	Maturity score
NVE	Night vision equipment
O₂	Oxygen
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RMT	Rectangular mid-water trawl (net)
RSV	Research and scientific vessel

Acronym	Definition
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acid
sp.	Species
TAG	Triacylglycerol
TLC-FID	Thin-layer chromatography-flame ionization detector
TL	Total length of the krill (Standard length 1)
TL.f	Total length as a factor, with classes of <31,31-33,33-35, 35-37, 37-39, >39mm
TSE	Total solvent extract
UL	Uropod length (mm)
µL	Micro-Litre
WAV	Within-animal variance
WE	Wax ester